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Neutralization of HIV-1 Subtypes: Implications for Vaccine Formulations

Tracey-Lee Smith, Estrelita J. van Rensburg, and Susan Engelbrecht*
Department of Medical Virology, University of Stellenbosch and Tygerberg Hospital, Tygerberg, South Africa

More than 20.8 million people are infected with HIV in sub-Saharan Africa, with South Africa having one of the fastest growing HIV-1 epidemics, where an estimated 2.4 million people were infected. Thirty-two sera from 25 patients were tested for their ability to neutralize HTLV-III_B (IIIB) and four primary isolates representing subtypes B, C, D, and a recombinant *gag* C/*env* B type. A CEM-SS cell line-based assay was used and the neutralizing titer was defined as the reciprocal of the highest dilution giving a 50% reduction in p24 antigen production. All isolates were neutralized better by subtype-specific sera, except for the C4714 strain, which was neutralized by both subtype B and C sera. C4714 was neutralized by 18/25 (72%) sera, IIIB by 19/32 (59%) sera, D482 by 7/31(23%) sera, B3245 by 6/29 (21%) sera, and the recombinant B/C1491 isolate by 4/25 (16%) sera. Five sera were unable to neutralize any of the isolates. The V3 region of the isolates used in the neutralization assay was amplified by PCR, directly sequenced, and analyzed to reveal variability between the consensus HIV-1 sequences and the isolates. HIV-1 strain C4714 was neutralized more effectively with the sera tested than the IIIIB laboratory strain. Variability in the amino acid sequence of the V3 region, which can alter the conformation of the V3 loop secondary structure, can influence the neutralization of a particular viral isolate. Vaccine formulations should be broadened to include multiple subtypes, especially C subtypes, which is rapidly spreading worldwide. ***J. Med. Virol. 56:264–268, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: South Africa; antibodies; PCR; V3 loop sequences

INTRODUCTION

More than 30 million people are infected with HIV, with 20.8 million living in sub-Saharan Africa [UNAIDS, 1997]. Currently, South Africa has one of the fastest growing HIV-1 epidemics, with an estimated 2.4 million people infected. Previous studies have identified three distinct subtypes circulating in South Africa: subtype C is found mainly in heterosexual groups, whereas subtypes B and D are found in the homosexual group. [Engelbrecht et al., 1995; Williamson et al., 1995; Van Harmelen et al., 1997].

Most vaccine formulations are derived from HIV-1 subtype B strains [Graham and Wright, 1995]. An important question to ask is whether or not a single subtype vaccine will be effective against all HIV-1 strains. More attention should be placed on the development of candidate vaccines against the other HIV-1 subtypes, especially subtype C, which is currently the cause of the majority of HIV-1 infections in developing countries, where it continues to spread extremely rapidly not only in southern Africa [Engelbrecht et al., 1995; Williamson et al., 1995; Van Harmelen et al., 1997] but also in India [Dietrich et al., 1993] and China [Luo et al., 1995]. Very few neutralization studies have used subtype C isolates or serum samples. This study was carried out using serum and viral isolates representing subtypes B, C, D, and a recombinant B/C subtype from South Africa in a CEM-SS cell line-based neutralization assay.

MATERIALS AND METHODS

Sera

Since 1984, virus was routinely isolated, DNA extracted, and serum collected from HIV-1–infected patients at hospitals in the Western Cape. A total of 32 sera from 25 HIV-1 ELISA antibody-positive individuals, most of whom had AIDS, were selected to test for the presence of neutralizing antibodies to five HIV-1 isolates. A direct V3 peptide-binding enzyme immunoassay (kindly supplied by Dr. E. Faatz, Boehringer Mannheim, Penzberg, Germany) was used to serotype 10 of the serum samples used. The other subtypes had

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*Correspondence to: Susan Engelbrecht, Department of Medical Virology, University of Stellenbosch and Tygerberg Hospital, P.O. Box 19063, Tygerberg, 7505, South Africa. E-mail: se1@gerga.sun.ac.za

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been identified previously by sequencing of a 1.5 kb region of the *env* gene and a 1.2 kb region of the *gag* gene of the corresponding isolates [Engelbrecht et al., 1995; Williamson et al., 1995].

Virus Isolates

The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: H9 infected with HTLV-III_B from Dr. Robert Gallo and CEM-SS cells from Dr. Peter Nara. Four additional isolates, B3245 and D482 [Engelbrecht et al., 1995], C4714 (Nof) [Dietrich et al., 1993], and a recombinant subtype B/C 1491 (*env* B and *gag* C) (data not shown) were isolated from patients' peripheral blood lymphocytes (PBLs) and cocultured using cord blood lymphocytes as described previously [Becker et al., 1985]. All the isolates were of the SI phenotype. Virus stocks were prepared in CEM-SS cells. Cultures were monitored for mycoplasma contamination [Van Kuppeveld et al., 1992]. The median tissue culture infectious dose ($TCID_{50}$) of the virus suspensions was determined prior to neutralization [Johnson and Byington, 1990].

Neutralization Assays

Neutralization was undertaken as described previously [Mascola et al., 1994; Nyambi et al., 1995]. The neutralizing titer of a particular serum and virus was defined as the reciprocal of the highest dilution giving a 50% reduction in p24 antigen production compared with the negative controls [Mascola et al., 1994]. HIV-1 serum neutralizing titers of 1:8 or greater were considered positive. The controls used were wells with cells only, virus only, cells and virus with and without seronegative sera, and cells and virus with pooled seropositive sera. The pooled seropositive sera was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH from Dr. Luba Vujcic [Vujcic and Quinnan, 1995]. To ensure the reproducibility of the assay, about 50% of the sera were randomly retested.

Polymerase Chain Reaction and Direct Sequencing of V3 Loop

Cell lysates were prepared from infected CEM-SS cells [Albert and Fenyö, 1990]. A heminested PCR was carried out with primer pairs ES7/ES8 and ES7/E125 (5′-CCTCAGGAGGGGACCCAATTG) [Sanders-Buell et al., 1995]. The ES7 and ES8 primers and the PCR method are described in the heteroduplex mobility analysis HIV-1 *env* subtyping kit obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. PCR products were visualized with ultraviolet light after electrophoresis through 2% agarose gels and ethidium bromide staining. The 337 bp product was directly sequenced using the Sequenase PCR product sequencing kit (Amersham, Arlington Heights, IL).

RESULTS

Neutralization Assays

The five virus isolates varied in their susceptibility to neutralization by the panel of sera tested (Table I). C4714 was neutralized well by most of the subtype B and C sera. The B3245 and D482 isolates were neutralized best by their type-specific sera. The B/C1491 recombinant isolate was only neutralized by its own sera and by 3/9 subtype B sera. The IIIB isolate was the only one neutralized by both B/C subtype sera. Neutralization of B3245 with subtype B sera was significantly poorer than IIIB (30% vs. 90%). Overall, C4714 was neutralized by 72% of the sera, the IIIB isolate by 59%, and the recombinant isolate by 16% (Table II).

PCR and Sequencing

A comparison of the V3 loop amino acid sequences of the isolates before culturing, i.e., PBL DNA from the initial isolations [Engelbrecht et al., 1995], and after culturing in CEM-SS cells, as well as consensus sequences from the Los Alamos database [Myers et al., 1996], is shown in Table III.

DISCUSSION

In accordance with other studies [Cheng-Mayer et al., 1988; Mascola et al., 1994], we are able to confirm that neutralization serotypes did correlate with genetic subtypes because the virus isolates were relatively more sensitive to neutralization by their subtype-specific sera, except isolate C4714, which was neutralized by both subtype B and C sera. In fact, C4714 was neutralized better than the two B subtype isolates, one of which was the laboratory strain.

A number of groups have observed a discrepancy between the neutralization of primary isolates and cell line-adapted strains [Wrin et al., 1995; Mascola et al., 1996; Moore et al., 1996; Moog et al., 1997], namely, the primary isolates are more resistant to neutralization than their laboratory-adapted strains.

Recently, it was found that syncytium-inducing (SI) virus strains that infect primary CD4 lymphocytes and transformed T-cell lines (T-tropic strains) use the CXCR4 coreceptor, while non–syncytium-inducing (NSI) strains that replicate in primary lymphocytes and macrophages (M-tropic strains) typically use the CCR5 coreceptor [Deng et al., 1996; Dragic et al., 1996]. Therefore, this assay, as well as others using T-cell lines, are limited to variants using the CXCR4 coreceptor i.e., SI viruses [Moore and Trkola, 1997]. Follow-up neutralization studies will be carried out using PBL's in order to ensure the isolation of both NSI and SI viruses.

From the comparison of the V3 amino acid sequences in Table III, it is clear that considerable variation exists between the consensus HIV-1 sequences and the isolates used in this study. The V3 loop amino acid changes between the isolates prepared in PBLs and those passaged in the CEM-SS T-cell line were conserved in most cases, with only two substitutions noted

TABLE I. Neutralization of Five Viral Isolates Using Different Serum Samples[a]

Sera	HIVIIIB	Isolate B3245	Isolate C4714	Isolate D482	Mosaic B/C1491
Control negative	<8	<8	<8	<8	<8
Control positive (B)	256	<8	<8	<8	128
B subtype					
B1(08/1986)	32	<8	32	<8	<8
B1(10/1987)	64	<8	64	<8	<8
B2	16	<8	8	<8	<8
B3	16	<8	8	<8	<8
B4[b]	128	<8	8	<8	8
B5[b]	<8	<8	<8	<8	8
B6[b]	64	8	32	8	<8
B7[b]	32	32	32	<8	<8
B8[b]	8	<8	16	64	32
B3245	32	16	ND	ND	ND
C subtype					
C1	64	8	128	16	<8
C2	8	<8	256	<8	<8
C3(03/1989)	8	<8	<8	<8	<8
C3(04/1991)	<8	ND	ND	<8	ND
C4714	<8	<8	8	<8	<8
C5	32	8	16	<8	<8
C6[b]	<8	<8	<8	<8	<8
C7[b]	<8	<8	8	<8	<8
C8[b]	<8	<8	64	<8	<8
D subtype					
D1	<8	<8	<8	<8	<8
D2(06/1985)	<8	<8	<8	<8	<8
D2(04/1986)	<8	ND	ND	8	ND
D3(04/1985)	<8	<8	ND	16	ND
D3(12/1987)	8	8	ND	32	ND
D3(11/1990)	64	<8	16	8	<8
D3(05/1991)	<8	<8	ND	<8	ND
D4	16	<8	<8	<8	<8
D482	<8	ND	ND	<8	ND
B/C subtype					
B/C1491(11/1986)	32	<8	<8	<8	16
B/C1491(03/1987)	16	<8	8	<8	<8
Mixed subtype					
A/B/C1[b]	<8	<8	1024	<8	<8
A/B/C2[b]	64	<8	128	<8	<8

[a]Reciprocal of highest serum dilution that produced 50% or more inhibition of in vitro HIV-1 p24 antigen production relative to control cultures. ND denotes not done.
[b]Sera typed with a V3 peptide ELISA.

TABLE II. Summary of Neutralization per Subtype

	Viral isolates (% neutralization)					% serum neutralization against all isolates
Sera	HIVIIIB	B3245	C4714	D482	B/C1491	
B	9/10 (90)	3/10 (30)	8/9 (89)	2/9 (22)	3/9 (33)	52.8
C	4/9 (44)	2/8 (25)	6/8 (75)	1/9 (11)	0/8 (0)	31
D	3/9 (33)	1/7 (14)	1/4 (25)	4/9 (44)	0/4 (0)	23
B/C	2/2 (100)	0/2 (0)	1/2 (50)	0/2 (0)	1/2 (50)	[a]
M	1/2 (50)	0/2 (0)	2/2 (100)	0/2 (0)	0/2 (0)	[a]
Total	19/32 (59)	6/29 (21)	18/25 (72)	7/31 (23)	4/25 (16)	

[a]Values were not determined because only two sera were tested.

in B/C1491 and C4714 and no change occurring in D482. The B3245 CEM-SS isolate, however, had five substitutions and an additional deletion compared to the original PBL isolate. C4714, which was the best neutralized antigen, shows the least variation when compared to the consensus 1996 and C consensus 1996 sequences [Blouin et al., 1996]. Amino acid changes, especially insertions or deletions, proximal to the tip can influence the specificity of neutralizing antibodies because they may alter the overall conformation of the

TABLE III. Amino Acid Sequence Alignment of *env* V3 Region: Comparison of Subtype Consensus Sequence and Viral Isolates Used in This Study[a]

All consensus 96	C	T	R	P	N	N	N	T	R	K	S	I	H	I	.	.	.	G	P	G	Q	A	F	Y	A	T	G	D	I	I	G	D	I	R	Q	A	H	C
B consensus 96	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	R	*	*	*	T	*	*	E	*	*	*	*	*	*	*	*	*	*
IIIB	*	*	*	*	*	*	*	*	*	*	R	*	*	*	Q	R	*	*	*	*	R	*	*	V	T	I	*	K	I	.	*	N	M	*	*	*	*	*
BC1491 (PBL)	*	*	*	*	*	*	*	*	*	*	*	*	R	*	G	H	I	*	*	*	R	*	L	*	T	*	*	R	*	*	*	*	*	*	*	*	*	*
BC1491 (CEM)	*	*	*	*	*	*	*	*	*	*	R	*	*	*	G	H	I	*	*	*	R	*	*	*	T	*	*	R	*	*	*	*	*	*	*	*	*	*
B3245 (PBL)	*	*	*	*	*	*	*	*	*	R	C	*	Y	*	*	*	*	*	*	*	R	*	*	H	T	.	.	.	*	Y	*	*	*	*	*	*	*	*
B3245 (CEM)	*	*	*	*	*	*	*	*	*	R	*	L	*	*	*	*	*	*	*	*	R	*	*	*	.	.	.	.	*	V	*	*	*	*	*	*	*	*
C consensus	*	*	*	*	*	*	*	*	*	*	*	*	R	*	*	*	*	*	*	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
C4714 (PBL)	*	*	*	*	*	*	*	*	*	*	R	*	*	V	*	*	*	*	*	*	*	T	V	*	*	*	N	A	*	*	*	*	*	*	*	*	*	*
C4714 (CEM)	*	*	*	*	*	*	*	*	*	*	R	*	R	V	*	*	*	*	L	*	*	T	V	*	*	*	N	A	*	*	*	*	*	*	*	*	*	*
D consensus	*	*	*	*	Y	*	*	*	*	Q	R	T	*	*	*	*	*	*	*	*	*	*	L	*	T	*	*	R	*	*	*	*	*	*	*	*	*	*
D482 (PBL)	*	*	*	*	Y	E	I	R	I	Q	K	T	S	*	*	*	*	*	Q	*	*	*	L	N	T	N	K	R	*	*	*	*	*	*	*	*	N	*
D482 (CEM)	*	*	*	*	Y	E	I	R	I	Q	K	T	S	*	*	*	*	*	Q	*	*	*	L	N	T	N	K	R	*	*	*	*	*	*	*	*	N	*

[a]Engelbrecht et al. [1995]; Myers et al. [1996]. . indicates amino acid deletion; *, concurrence with top sequence.

loop. Therefore, it can be expected that the variation described in these isolates could have contributed to the poor neutralization of these three strains. With these data in mind, it may be expected that neutralization with different descendants of a stock virus could yield different results. This is important not only when comparing experimental results within the laboratory, but also when comparing results with those of other laboratories.

In addition to the V3 loop, other neutralizing sites include epitopes in the C2 [Ho et al., 1988] and V2 [Moore et al., 1993] regions of gp120, as well as in gp41 [Dalgleish et al., 1988], and a conformational epitope that overlaps the CD4 binding region of the envelope glycoprotein. Escape from neutralizing antisera may also occur through mutations in one of these epitopes and influence the binding of different antibodies to numerous sites on the protein.

In conclusion, the results indicate that subtype B strains may not be the best neutralizing antigen for vaccine development. A broadening of vaccine development efforts must be considered to include multiple subtypes, especially subtype C, to control the global spread of HIV-1.

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